Association of Apolipoprotein E Polymorphism with Ischemic Stroke Subtypes in Taiwan

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The aim of this study was to clarify whether the apolipoprotein E gene (*APOE*) is related to ischemic stroke subtypes in Taiwan's Chinese population. Using the classification of Cerebrovascular Diseases III, 143 patients with lacunar infarction, 114 patients with atherothrombotic infarction, and 112 healthy controls were enrolled. *APOE* genotype was determined using polymerase chain reaction. Regarding the distribution of *APOE* genotypes, the frequency of ε3/ε4 genotypes in lacunar patients was significantly different from that in control subjects, by logistic regression, using ε3/ε3 as a reference group. There was no significant difference between atherothrombotic patients and the control group in the distribution of *APOE* genotypes or alleles. The present finding suggests that there is a probable association between ε3/ε4 genotype and lacunar infarcts, but not atherothrombotic infarcts. This indicates that genetic factors may play a role, at least partially, in lacunar infarction in Taiwan's Chinese population.

Key Words: apolipoprotein E, ischemic stroke, Taiwan
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Ischemic stroke is a major cause of morbidity and mortality in the elderly. Although several vascular risk factors [1,2], such as hypertension, diabetes mellitus, cigarette smoking and alcohol consumption, have been established, the role of other putative risk factors like hypercholesterolemia still needs to be elucidated. On the other hand, the types of ischemic stroke seem to differ between Western and Asian countries, and Asians have been found to have more intracranial atherosclerosis than Caucasians [3–7]. In the Western population, a positive association between cholesterol levels and ischemic stroke has been noted [8], whereas in studies on Asians, an inverse relationship between cholesterol levels and the occurrence of stroke has been observed [3,9].

Apolipoprotein E (*APOE*; NM_000041) is an important regulator of the metabolism of cholesterol and other lipids [10]. *APOE* has three major isoforms, *APOE*2, *E*3, and *E*4, which are encoded by three alleles (epsilon 2, 3, and 4) at a gene located on the long arm of chromosome 19. The *E*2 (MIM 107741.0001), *E*3 (MIM 107741.0015) and *E*4 (MIM 107741.0016) isoforms differ in amino acid sequence at two sites: residue 112 (called site A) and residue 158 (called site B). At sites A/B, *APOE*2, *E3* and *E*4 contain cysteine/cysteine, cysteine/arginine and arginine/arginine, respectively. ε4 has been found to be associated with higher plasma cholesterol levels and ε2 with lower plasma cholesterol levels [11]. However, the association of *APOE* with stroke has been controversial [1,2,9,12–19], possibly due to inaccurate classifications

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of stroke or small sample sizes. On the other hand, the distributions of *APOE* alleles have been found to have wide variations among different populations [20–29]. For example, studies have shown that the frequency of the *APOE* ε4 allele ranges from 9.0% to 16.5% in Western populations and from 4.9% to 11.0% in Chinese [22–24,26,28,29]. Whether the difference in the *APOE* polymorphism between races is an independent predictor of intracranial atherosclerotic stroke or whether it is confounded by the prevalence of various stroke risk factors has not been clearly determined. Thus, the aim of this study was to clarify the association of the *APOE* polymorphism with ischemic stroke subtypes in Taiwan Chinese.

Patients and Methods

The present case-control study was conducted in Kaohsiung Medical University Hospital (KMUH), Taiwan, from January 1996 to December 1997. Patients with rheumatic heart disease, epidural hematoma, brain tumor, or accidental or iatrogenic stroke were excluded from the study. Of the 406 consecutive patients with ischemic stroke and neurologic symptoms lasting more than 24 hours, accompanied by corresponding focal density changes detected by magnetic resonance imaging during the study period, 326 (80.4%) were examined at the neurologic outpatient clinic 3 months after the stroke. The remaining 80 patients were not enrolled in the present study (including 53 who had moved, lost contact, or refused follow-up evaluation, 12 who had incomplete data collection, 10 who had died prior to the first follow-up and 5 who lived in long-term care institutions). Of the 326 patients, 49 cases with probable vascular dementia according to the National Institute of Neurological Disorders and Stroke and the Association International pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) clinical criteria were excluded to avoid classification confusion with mixed-type dementia. Ischemic stroke was classified as lacunar infarction and atherothrombotic infarction according to the Classification of Cerebrovascular Diseases III [30]. Under this classification, 20 cases that belonged to the category of ischemic stroke with cardioembolism or other types were also excluded. Finally, 143 cases were defined as having lacunar infarcts and 114 cases as having atherothrombotic infarcts.

Control subjects ($n = 112$) were healthy elderly volunteers who underwent a regular health examination in KMUH in 1996. Each healthy control subject was interviewed and examined by a neurologist to rule out stroke and cognitive deficits clinically. There were no relations of kinship among the controls and/or stroke patients.

Genomic DNA was extracted from peripheral blood leukocytes using a QIAmp blood kit (QIAGEN Inc., Valencia, CA, USA) after informed consent had been obtained. Exon 4 of the *APOE* gene was amplified by polymerase chain reaction (PCR) using the upstream primer 5′-TCGCGGGCCCCCGGGCCTGGTACA-3′ and the downstream primer 5′-ACAGAATTCGCCC-CGGCCTGGTACACTGCCA-3′. The PCR products were digested with HhaI, and the fragments were separated by electrophoresis on a 6% polyacrylamide gel, followed by ethidium bromide staining. DNA fragments were visualized under ultraviolet illumination. The *APOE* genotypes of patient and healthy control groups were determined in a blinded fashion by scoring for a unique combination of fragment sizes, as described by Wenham et al [31].

Statistical analysis

A two-tailed Student's *t* test was used to compare ages and cholesterol levels between patients and the healthy control group. Comparisons of lacunar patients and atherothrombotic patients with controls were conducted using the χ^2 test on the frequencies of sex, hypertension and diabetes mellitus (DM). The allele frequencies in lacunar and atherothrombotic infarction patients and control subjects were estimated by counting alleles and calculating sample proportions. Comparisons of the genotype and allele frequencies were made using a logistic regression model. The odds ratios (ORs) were compared for the association of *APOE* ε4 in the healthy controls (ε3/ε3) and lacunar or atherothrombotic infarction patients.

Results

The mean ± standard deviation age was 64.3 ± 7.9 years for lacunar infarct patients, 63.0 ± 8.6 years for atherothrombotic patients, and 71.0 ± 10.6 years for healthy controls. There were statistically significant differences in age, with a preponderance of older ages in the controls. The lacunar group consisted of 91 (63.6%) males

Table 1. Clinical characteristics of Taiwan Chinese patients with ischemic cerebrovascular diseases and control subjects*

	Lacunar patients ($n=143$)	Atherothrombotic patients ($n=114$)	Controls ($n=112$)
Age (yr)	64.3 ± 7.9	63.0 ± 8.6	71.0 ± 10.6
Sex (M/F)	91/52	73/41	54/58
Hypertension	111 (77.6)†	93 (81.6)†	31 (27.7)
Diabetes mellitus	55 (38.5)†	47 (41.2)†	15 (13.4)
Cholesterol (mg/dL)	206.4 ± 45.0	199.8 ± 41.5	202.0 ± 33.8

*Data are presented as mean ± standard deviation or *n* (%); †$p < 0.05$ *vs.* control subjects, χ^2 test.

Table 2. Distribution of apolipoprotein E genotypes and alleles in Taiwan Chinese patients with ischemic cerebrovascular diseases and control subjects*

	Lacunar patients ($n=143$)	Atherothrombotic patients ($n=114$)	Controls ($n=112$)	Lacunar patients *vs.* controls, *p*	Atherothrombotic patients *vs.* controls, *p*
ε2	14 (4.9)	15 (6.6)	18 (8.0)	0.204	0.576
ε3	224 (78.3)	184 (80.7)	180 (80.4)	–	–
ε4	48 (16.8)	29 (12.7)	26 (11.6)	0.134	0.763
ε2/ε2	0	1 (0.9)	4 (3.6)	–	0.224
ε2/ε3	10 (7.0)	7 (6.1)	5 (4.5)	0.287	0.565
ε2/ε4	4 (2.8)	6 (5.3)	5 (4.5)	0.654	0.755
ε3/ε3	85 (59.4)	77 (67.5)	78 (69.6)	–	–
ε3/ε4	44 (30.8)	23 (20.2)	19 (16.9)	0.017†	0.559
ε4/ε4	0	0	1 (0.9)	–	–

*Data are presented as *n* (%); †$p < 0.05$ *vs.* control subjects, logistic regression using ε3/ε3 as a reference group.

and 52 (36.4%) females. The atherothrombotic group consisted of 73 (64.0%) males and 41 (36.0%) females, and the control group was made up of 54 (48.2%) males and 58 (51.4%) females. The lacunar and atherothrombotic groups showed a significant male predominance (Table 1).

As shown in Table 1, the frequencies of personal histories of hypertension and DM were significantly higher in lacunar and atherothrombotic infarct patients. There were no significant differences between ischemic stroke patients and healthy controls in terms of cholesterol level.

There was no significant difference in *APOE* genotype frequency between male and female subjects. The *APOE* allelic frequencies of the lacunar patients were as follows: ε2, 4.9%; ε3, 78.3%; ε4, 16.8%. In the healthy control subjects, the data were as follows: ε2, 8.0%; ε3, 80.4%; ε4, 11.6%. The allele frequencies among the three groups were not significantly different (Table 2). However, comparison of the genotype distribution, by logistic regression using ε3/ε3 as the reference group, showed a statistically significant difference in the frequency of ε3/ε4 genotypes in patients with lacunar infarcts (Table 2). Compared with healthy control subjects, the OR of ε3/ε4 in lacunar patients was 2.13 (95% confidence interval, 1.16–4.02; $p = 0.017$).

The allelic frequencies of *APOE* in the atherothrombotic group were 6.6% for ε2, 80.7% for ε3 and 12.7% for ε4 (Table 2). No significant difference in the ε4 or ε2 allele frequencies was noted between atherothrombotic patients and healthy control subjects. There was also no significant difference between atherothrombotic patients and the control group in terms of genotype distribution, by logistic regression, using ε3/ε3 as a reference group.

Discussion

In the present study, the ε3/ε4 genotype frequencies in lacunar patients were significantly higher than those in healthy controls (30.8% *vs.* 16.9%). The odds of carrying a ε3/ε4 genotype in lacunar patients is twice that in control subjects (OR, 2.13), by logistic regression using ε3/ε3 as a reference group. This finding suggests that there may be a greater risk of lacunar

infarct in patients with the ε3/ε4 genotype. Previously, the ε3/ε4 genotype has been associated with more severe atherosclerosis [32] and may be related to the development of carotid stenosis [19]. It is worth noting that although lacunar infarcts are generally supposed to be caused by hypertensive small-vessel disease [12,33,34], other etiologies, including middle cerebral artery atherosclerotic disease or hemodynamic compromise from a carotid lesion, have been postulated to be related to lacunar infarcts [35]. In a study of healthy families, it has been found that genetic factors may strongly contribute to the intima-media thickness variability and that the *APOE* polymorphism may be one of these factors [36]. Thus, it seems that lacunar infarct may be related to a diversity of etiopathogenic mechanisms, which may include the *APOE* polymorphism. However, it remains to be determined whether genetic or environmental factors, or their interaction, can explain the heterogeneity in lacunar infarction risk in subjects with the ε3/ε4 genotype.

According to a previous report [3], deep cerebral infarcts (lacunar infarct) were more common in Chinese patients and are usually larger than those seen in patients from the West. This difference in lacunar presentation could have been due to the effects of various genetic factors. However, regarding the allele frequency, although a higher prevalence of ε4 carriers in lacunar patients has been described in some previous studies [1,9,12,19], this is not the case in the present study (16.8% *vs.* 11.6%, no significant difference). This may have been due to the fact that ischemic stroke is a heterogeneous disease and the classification of ischemic stroke into lacunar and atherothrombotic subtypes with the exclusion of cardioembolic stroke may have allowed differences to emerge [12].

On the other hand, atherosclerosis in larger arteries has been related to high lipid levels and hypertension [1,37]. Because the *APOE* polymorphism plays a key role in plasma lipid level regulation [10], it is assumed that *APOE* isoforms play an important role in the pathogenesis of atherosclerosis [38]. However, the present study demonstrates no significant differences in *APOE* ε4 or ε2 allele frequencies between patients with atherothrombotic infarction and control subjects, and the difference in genotype distribution between the two groups is also insignificant (Table 2). This suggests that the ε4 allele is not significantly related to the development of atherothrombotic infarction and that the ε2 allele is not a significant factor in preventing the development of atherothrombotic infarction, in line with some previous reports [14,16,17]. Therefore, the association of the *APOE* polymorphism with atherothrombotic infarction remains controversial, and other risk factors of atherothrombotic infarct will be sought in the future.

Nonetheless, there are some significant points of interest in the present study. First, although the present study focused on the genetic risk factors for ischemic stroke, some common stroke risk factors were also evaluated and analyzed. It was shown that the frequencies of personal histories of hypertension and DM were significantly higher in lacunar and atherothrombotic infarct patients. These findings are consistent with previous studies [6,7], which found that hypertension and DM are risk factors for atherosclerosis. It is interesting to note that although the role of apolipoprotein E polymorphism in the development of ischemic infarcts in patients with DM has not been investigated [39], the *APOE* ε4 allele has been found to be a susceptibility locus for systemic hypertension and carotid artery atherosclerosis [40]. On the other hand, there was no major association with cholesterol metabolism in stroke patients in the present study. Therefore, the relationship between cholesterol metabolism and stroke occurrence is still unclear. Second, some clinical variables, such as the carotid status [41], incidences of smoking and alcohol consumption [40], and history of previous transient ischemic attacks [42], were not measured in the present study, and these factors might confound the association of *APOE* polymorphism with ischemic stroke. Third, the relatively small sample size limited the power to stratify coexisting risk factors (such as hypertension and DM) in both lacunar and atherothrombotic infarction. The associations between the *APOE* polymorphism and hypertension, DM or cholesterol level in ischemic stroke patients need further epidemiologic studies.

In conclusion, the present study of Taiwan Chinese stroke patients demonstrates a probable association between ε3/ε4 genotype and lacunar infarction, but not between *APOE* genotype or allele frequency and atherothrombotic infarction. Although the pathogenesis of lacunar infarction remains unclear, the present study suggests that genetic factors may play a role, at least partially, in the occurrence of lacunar infarction. Further studies are necessary to clarify the confounding factors in ischemic stroke, such as hypertension and DM.

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台灣之 Apolipoprotein E 基因多形性與缺血性腦中風類型之相關性研究

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本研究之目的是探討在台灣 apolipoprotein E (APOE) 基因多形性與缺血性腦中風類型之相關性。我們採用 cerebrovascular disease III 之分類，徵召 143 位腔隙型缺血性腦中風患者，114 位粥狀梗塞型缺血性腦中風患者，及 112 位正常人。利用血液中之白血球，作 APOE 基因多形性分析。結果發現，腔隙型缺血性腦中風患者具 ε3/ε4 之比率呈現有意義的差別。但在粥狀梗塞型缺血性腦中風患者不論是基因型或其對偶基因分佈，都無顯著差異。本研究結果顯示，ε3/ε4 之基因型可能與腔隙型缺血性腦中風有關，而粥狀梗塞型缺血性腦中風則否。因此我們認為，對罹患腔隙型缺血性腦中風之在台灣的中國人，APOE 基因扮演著部分的角色。

關鍵詞：apolipoprotein E，缺血性腦中風，台灣

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